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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
O FARRELL, THOMAS JOHN	

ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 02/15/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/727,664	YAKU ET AL.	
	Examiner	Art Unit	
	Thomas J. O'Farrell	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 November 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-22* is/are pending in the application.

4a) Of the above claim(s) *23-36* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-22* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *07 May 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *12/05/2003*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election without traverse of group 1, claims 1-22 in the reply filed on 11/17/2005 is acknowledged.

2. Claims 1-22 are currently under consideration. An action on the merits follows. Claims 23-36 are withdrawn from consideration as being drawn to non elected inventions.

Priority

3. Acknowledgment is made of applicant's claim for foreign priority based on applications filed in Japan on 12/06/2002 and 08/07/2002. It is noted, however, that applicant has not filed certified translated copies of the 2002-355915 and 2003-288707 applications. Therefore, priority to the 2002-355915 and 2003-288707 applications has not been granted to the instantly pending claims.

Double Patenting

4. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the

unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., In re Berg, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); In re Goodman, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); In re Longi, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); In re Van Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and In re Thorington, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

5. Claims 1-9, 14-17 and 19 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 3, 4, and 6 of copending Application No. 11180881, in view of Sorenson (herein referred to as Sorenson, WO 93/22456, 11/1993), and Kambara et al. (herein referred to as Kambara, JP 2002-101899, 04/09/2002). Although the conflicting claims are not identical, they are not patentable distinct from each other because the claims are coextensive in scope.

Claims 1, 3, 4, and 6 of the '881 application teach methods of measuring pyrophosphate by adding pyrophosphate to a two region membrane system that contains pyrophosphatase and measuring the pH in either region, where pH could be measured optically by a pH sensitive dye or measured electrically.

Claims 1, 3, 4, and 6 of the '881 application do not teach methods of detecting mutations where sequence where a target DNA is amplified by PCR with primers that have a different base at their 3' nucleotide corresponding to the possible mutations that can occur at the position in the target corresponding to the 3' nucleotide of the primer. However, Sorenson teaches a method to detect a mutant gene sequence where a target DNA is amplified by PCR with primers that have a different base at their 3' nucleotide corresponding to the possible mutations that can occur at the position in the target corresponding to the 3' nucleotide of the primer (claims 1, 3 and 4; see all of para 3 on page 3 and para 1 on page 4 of Sorenson). Sorenson teaches that the presence of a amplified polynucleotide indicates the presence of the specific base pair in the target corresponding to that in the allele specific primer and this amplified DNA can be analyzed by electrophoresis with ethidium bromide staining (claims 1, 5, and 6, see page 4, lines 1-3 of para 2, and page 6, lines 1-4 of Sorenson). Sorenson teaches that the allele specific primers can have some mismatches 3-6 nucleotides from the 3' end and the primers can be 16 bases in length (claim 1; see page 16, all of para 1 and Table 1 of Sorenson). Sorenson teaches that the polymerase used for the amplification method above can lack 3' exonuclease activity (claim 2; see page 5, all of para 2 of Sorenson). Sorenson teaches that the primers used in the above procedure can be labeled to permit quantitation of the amplified product (claim 9, see page 6, para 1, lines 4-11 of Sorenson). Sorenson teaches that two or more allele specific primers of different lengths can be used in the same amplification reaction where the length of the

amplified polynucleotide would indicate which mutation was present (claims 20 and 21; see page 16, all of para 2 of Sorenson).

Sorenson does not teach a method of mutation detection where the allele specific primers have an uncomplimentary region which is located adjacent to the substitution corresponding region on a 5' terminal side thereof and consists *specifically of two bases* uncomplimentary to the target DNA (claims 1 and 20). However, Sorenson teaches that the allele specific primers can have some mismatches 3-6 nucleotides from the 3' end that would not be likely to interfere with efficacy (see page 16, para 1, lines 1-3 of Sorenson). In addition, per MPEP 2144.05, where the general conditions of the claim are disclosed in the prior art, it is not inventive to discover optimum or workable ranges by routine experimentation and it is the normal desire of scientists and artisans to improve on what is already generally known. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made improve the method of mutation detection taught by Sorenson through routine experimentation to provide optimal or workable ranges, such as where the uncomplimentary region of the primer consists *specifically of two bases* uncomplimentary to the target DNA, in view of Sorenson. The ordinary artisan would have been motivated to improve the method of mutation detection taught by Sorenson through routine experimentation to provide optimal or workable ranges, such as where the uncomplimentary region of the primer consists *specifically of two bases* uncomplimentary to the target DNA, for the purpose of obtaining the optimal range to practice the method of mutation detection taught by Sorenson.

Sorenson does not teach a method of mutation detection involving allele specific amplification *specifically where the progress or amount of product of the reaction is analyzed by measuring the amount of pyrophosphate generated by the primer extension reaction.* However, Kambara teaches that primer extension reactions produce large amounts of pyrophosphate that can be detected by chemiluminescence to attain a highly sensitive detection of base mutations (see para 0030 of translation of Kambara).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of mutation detection taught by Sorenson by analyzing the progress or amount of product of the reaction by detecting the pyrophosphate generated by the primer extension reaction in view of the teachings of Kambara. The ordinary artisan would have been motivated to improve the method of mutation detection taught by Sorenson by analyzing the progress or amount of product of the reaction by detecting the pyrophosphate generated by the primer extension reaction because Kambara teaches that primer extension reactions produce large amounts of pyrophosphate that can be detected by chemiluminescence to attain a highly sensitive detection of base mutations.

It would have been further prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify claims 1, 3, 4, and 6 of the '881 application that teach an effective method of measuring pyrophosphate levels by adding pyrophosphate to a two region membrane system that contains pyrophosphatase and measuring the pH in either region, where pH could be measured optically by a pH

sensitive dye or electrically to include the measurement of the pyrophosphate produced from the amplification step taught in the method of mutation detection of Sorenson and Kambara to provide for a method of mutation detection using the method of claims 1, 3, 4, and 6 of the '881 application in view of the teachings of Sorenson and Kambara. The ordinary artisan would have been motivated to use the method of measuring pyrophosphate taught in claims 1, 3, 4, and 6 of the '881 application that teach a method of measuring pyrophosphate levels by adding pyrophosphate to a two region membrane system that contains pyrophosphatase and measuring the pH in either region, where pH could be measured optically by a pH sensitive dye or electrically to include the measurement of the pyrophosphate produced from the amplification step taught in the method of mutation detection because Sorenson and Kambara teach a method of measuring pyrophosphate levels that could be effectively used to determine base mutations.

This is a provisional obviousness-type double patenting rejection.

6. Claim 18 is provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 3, 4, and 6 of copending Application No. 11180881, in view of Sorenson and Kambara, as applied to claims 1-9, 14-17 and 19 above, further in view of Morad et al (herein referred to as Morad, US Patent 4894376, 01/1990). Although the conflicting claims are not identical, they are not patentable distinct from each other because the claims are coextensive in scope.

The teachings claims 1, 3, 4, and 6 of the '881 application, further in view of Sorenson, further in view of Kambara, as applied to claims 1-9, 14-17 and 19 are recited above in para 5.

Claims 1, 3, 4, and 6 of the '881 application, further in view of Sorenson, further in view of Kambara do not teach measuring pyrophosphate levels by measuring changes in pH in a membrane system containing pyrophosphatase *specifically where measuring changes in pH is conducted with a patch-clamp method.* However, Morad teaches that the patch-clamp technique allows precise measurement of current caused by ion flux through ion channels in cells. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of detecting pyrophosphate produced from the amplification step taught in the method of mutation detection taught by claims 1, 3, 4, and 6 of the '881 application, further in view of Sorrenson, further in view of Kambara by using a patch-clamp technique to measure the change in H+ concentration produced by pyrophosphate by exposure to a membrane containing pyrophosphatase in view of the teachings of Morad. The ordinary artisan would have been motivated to modify the method of detecting pyrophosphate produced from the amplification step taught in the method of mutation detection taught by claims 1, 3, 4, and 6 of the '881 application, further in view of Sorenson, further in view of Kambara, by using a patch-clamp technique to measure the change in H+ concentration produced by pyrophosphate by exposure to a membrane containing pyrophosphatase because Morad teaches that the patch-clamp

technique allows precise measurement of current caused by ion flux through ion channels in cells.

This is a provisional obviousness-type double patenting rejection.

7. Claims 1-13 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 2, 3, 5, and 6 of copending Application No. 10699848, in view of Sorenson and Kambara. Although the conflicting claims are not identical, they are not patentable distinct from each other because the claims are coextensive in scope.

Claims 2, 3, 5, and 6 of the '848 application teach methods of measuring pyrophosphate levels where the pyrophosphate is converted to phosphoric acid by pyrophosphatase, which is used in a catalysis step with glyceraldehyde 3-phosphatedehydrogenase plus additional reagents that are used in instant claims 11 and 12 to produce a current which is measured.

Claims 2, 3, 5, and 6 of the '848 application do not teach methods of detecting mutations where sequence where a target DNA is amplified by PCR with primers that have a different base at their 3' nucleotide corresponding to the possible mutations that can occur at the position in the target corresponding to the 3' nucleotide of the primer. However, Sorenson teaches a method to detect a mutant gene sequence where a target DNA is amplified by PCR with primers that have a different base at their 3' nucleotide corresponding to the possible mutations that can occur at the position in the target corresponding to the 3' nucleotide of the primer (claims 1, 3 and 4; see all of para

3 on page 3 and para 1 on page 4 of Sorenson). Sorenson teaches that the presence of a amplified polynucleotide indicates the presence of the specific base pair in the target corresponding to that in the allele specific primer and this amplified DNA can be analyzed by electrophoresis with ethidium bromide staining (claims 1, 5, and 6, see page 4, lines 1-3 of para 2, and page 6, lines 1-4 of Sorenson). Sorenson teaches that the allele specific primers can have some mismatches 3-6 nucleotides from the 3' end and the primers can be 16 bases in length (claim 1; see page 16, all of para 1 and Table 1 of Sorenson). Sorenson teaches that the polymerase used for the amplification method above can lack 3' exonuclease activity (claim 2; see page 5, all of para 2 of Sorenson). Sorenson teaches that the primers used in the above procedure can be labeled to permit quantitation of the amplified product (claim 9, see page 6, para 1, lines 4-11 of Sorenson). Sorenson teaches that two or more allele specific primers of different lengths can be used in the same amplification reaction where the length of the amplified polynucleotide would indicate which mutation was present (claims 20 and 21; see page 16, all of para 2 of Sorenson).

Sorenson does not teach a method of mutation detection where the allele specific primers have an uncomplimentary region which is located adjacent to the substitution corresponding region on a 5' terminal side thereof and consists *specifically of two bases* uncomplimentary to the target DNA (claims 1 and 20). However, Sorenson teaches that the allele specific primers can have some mismatches 3-6 nucleotides from the 3' end that would not be likely to interfere with efficacy (see page 16, para 1, lines 1-3 of Sorenson). In addition, per MPEP 2144.05, where the general

conditions of the claim are disclosed in the prior art, it is not inventive to discover optimum or workable ranges by routine experimentation and it is the normal desire of scientists and artisans to improve on what is already generally known. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made improve the method of mutation detection taught by Sorenson through routine experimentation to provide optimal or workable ranges, such as where the uncomplimentary region of the primer consists *specifically of two bases* uncomplimentary to the target DNA, in view of Sorenson. The ordinary artisan would have been motivated to improve the method of mutation detection taught by Sorenson through routine experimentation to provide optimal or workable ranges, such as where the uncomplimentary region of the primer consists *specifically of two bases* uncomplimentary to the target DNA, for the purpose of obtaining the optimal range to practice the method of mutation detection taught by Sorenson.

Sorenson does not teach a method of mutation detection involving allele specific amplification *specifically where the progress or amount of product of the reaction is analyzed by measuring the amount of pyrophosphate generated by the primer extension reaction*. However, Kambara teaches that primer extension reactions produce large amounts of pyrophosphate that can be detected by chemiluminescence to attain a highly sensitive detection of base mutations (see para 0030 of translation of Kambara).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of mutation detection taught by

Sorenson by analyzing the progress or amount of product of the reaction by detecting the pyrophosphate generated by the primer extension reaction in view of the teachings of Kambara. The ordinary artisan would have been motivated to improve the method of mutation detection taught by Sorenson by analyzing the progress or amount of product of the reaction by detecting the pyrophosphate generated by the primer extension reaction because Kambara teaches that primer extension reactions produce large amounts of pyrophosphate that can be detected by chemiluminescence to attain a highly sensitive detection of base mutations.

It would have been further prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify claims 2, 3, 5, and 6 of the '848 application that teach an effective method of measuring pyrophosphate levels where the pyrophosphate is converted to phosphoric acid by pyrophosphatase, which is used in a catalysis step with glyceraldehyde 3-phosphatedehydrogenase plus additional reagents that are used in instant claims 11 and 12 to produce a current which is measured to include the measurement of the pyrophosphate produced from the amplification step taught in the method of mutation detection of Sorenson and Kambara to provide for a method of mutation detection using the method of claims 2, 3, 5, and 6 of the '848 application in view of the teachings of Sorenson and Kambara. The ordinary artisan would have been motivated to use the method of pyrophosphate determination taught by claims 2, 3, 5, and 6 of the '848 application that teach a method of measuring pyrophosphate levels where the pyrophosphate is converted to phosphoric acid by pyrophosphatase, which is used in a catalysis step with glyceraldehyde 3-

phosphatedehydrogenase plus additional reagents that are used in instant claims 11 and 12 to produce a current which is measured to include the measurement of the pyrophosphate produced from the amplification step taught in the method of mutation detection because Sorenson and Kambara teach a method of measuring pyrophosphate levels that could be effectively used to determine base mutations.

This is a provisional obviousness-type double patenting rejection.

Claim Rejections - 35 USC § 112

8. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> **The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.**

9. Claims 1-22 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 1 and 20 recite the phrase "3' terminal". It is unclear whether "3' terminal" refers to the 1st nucleotide on the 3' end of the polynucleotide or whether it includes any nucleotide that has another nucleotide 5' to it.

Claim Rejections - 35 USC § 103

10. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

11. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

12. Claims 1-6, 9, 20 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sorenson.

Sorenson teaches a method to detect a mutant gene sequence where a target DNA is amplified by PCR with primers that have a different base at their 3' nucleotide corresponding to the possible mutations that can occur at the position in the target corresponding to the 3' nucleotide of the primer (claims 1, 3 and 4; see all of para 3 on page 3 and para 1 on page 4 of Sorenson). Sorenson teaches that the presence of a amplified polynucleotide indicates the presence of the specific base pair in the target corresponding to that in the allele specific primer and this amplified DNA can be analyzed by electrophoresis with ethidium bromide staining (claims 1, 5, and 6, see

page 4, lines 1-3 of para 2, and page 6, lines 1-4 of Sorenson). Sorenson teaches that the allele specific primers can have some mismatches 3-6 nucleotides from the 3' end and the primers can be 16 bases in length (claim 1; see page 16, all of para 1 and Table 1 of Sorenson). Sorenson teaches that the polymerase used for the amplification method above can lack 3' exonuclease activity (claim 2; see page 5, all of para 2 of Sorenson). Sorenson teaches that the primers used in the above procedure can be labeled to permit quantitation of the amplified product (claim 9, see page 6, para 1, lines 4-11 of Sorenson). Sorenson teaches that two or more allele specific primers of different lengths can be used in the same amplification reaction where the length of the amplified polynucleotide would indicate which mutation was present (claims 20 and 21; see page 16, all of para 2 of Sorenson).

Sorenson does not teach a method of mutation detection where the allele specific primers have an uncomplimentary region which is located adjacent to the substitution corresponding region on a 5' terminal side thereof and consists *specifically of two bases* uncomplimentary to the target DNA (claims 1 and 20). However, Sorenson teaches that the allele specific primers can have some mismatches 3-6 nucleotides from the 3' end that would not be likely to interfere with efficacy (see page 16, para 1, lines 1-3 of Sorenson). In addition, per MPEP 2144.05, where the general conditions of the claim are disclosed in the prior art, it is not inventive to discover optimum or workable ranges by routine experimentation and it is the normal desire of scientists and artisans to improve on what is already generally known. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the

invention was made improve the method of mutation detection taught by Sorenson through routine experimentation to provide optimal or workable ranges, such as where the uncomplimentary region of the primer consists *specifically of two bases* uncomplimentary to the target DNA, in view of Sorrenson. The ordinary artisan would have been motivated to improve the method of mutation detection taught by Sorrenson through routine experimentation to provide optimal or workable ranges, such as where the uncomplimentary region of the primer consists *specifically of two bases* uncomplimentary to the target DNA, for the purpose of obtaining the optimal range to practice the method of mutation detection taught by Sorenson.

13. Claims 7 and 8 rejected under 35 U.S.C. 103(a) as being unpatentable over Sorenson as applied to claims 1-6, 9, 20, and 21 above, and further in view of Kambara.

The teachings of Sorenson as applied to claims 1-6, 9, 20, and 21 are recited above in para 12.

Sorrenson does not teach a method of mutation detection involving allele specific amplification *specifically where the progress or amount of product of the reaction is analyzed by measuring the amount of pyrophosphate generated by the primer extension reaction.* However, Kambara teaches that primer extension reactions produce large amounts of pyrophosphate that can be detected by chemiluminescence to attain a highly sensitive detection of base mutations (see para 0030 of translation of Kambara). Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of mutation detection

taught by Sorenson by analyzing the progress or amount of product of the reaction by detecting the pyrophosphate generated by the primer extension reaction in view of the teachings of Kambara. The ordinary artisan would have been motivated to improve the method of mutation detection taught by Sorenson by analyzing the progress or amount of product of the reaction by detecting the pyrophosphate generated by the primer extension reaction because Kambara teaches that primer extension reactions produce large amounts of pyrophosphate that can be detected by chemiluminescence to attain a highly sensitive detection of base mutations.

14. Claim 22 is rejected under 35 U.S.C. 103(a) as being unpatentable over Sorenson as applied to claims 1-6, 9, 20, and 21 above, and further in view of Newton (herein referred to as Newton, US Patent 5525494, 06/1996).

The teachings of Sorenson as applied to claims 1-6, 9, 20 and 21 are recited above in para 12.

Sorenson does not teach a method of mutation detection involving allele specific amplification *specifically where the primers are labeled by their respective fluorenscences which are different in wavelength.* However, Newton teaches that allele specific amplification can be conveniently effected by labeling the primers with different fluorescent labels such as fluoroscein (green) and rhodamine (red) to allow the detection of homo- and heterozymgotes by color blending (see column 4, lines 54-64 of Newton. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of mutation detection

taught by Sorenson by labeling the primers used for primer extension with different fluorescent labels such as fluoroscein (green) and rhodamine (red) to allow the detection of homo- and heterozygotes by color blending in view of the teachings of Newton. The ordinary artisan would have been motivated to improve the method of mutation detection taught by Sorenson by labeling the primers used for primer extension with different fluorescent labels such as fluoroscein (green) and rhodamine (red) to allow the detection of homo- and heterozygotes by color blending because Newton teaches that this labeling method allows for convenient detection of mutations by allele specific amplification.

15. Claims 14-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sorenson further in view of Kambara as applied to claims 7 and 8 above, and further in view of Bille et al. (herein referred to as Bille, 1992, Phys. Plantarum, vol. 84, pages 250-254).

The teachings of Sorrenson further in view of Kambara as applied to claims 7 and 8 are recited in para 13 above.

Sorenson further in view of Kambara do not teach a method of mutation detection using amplification and measurement of pyrophosphate *specifically where the pyrophosphate is detected by applying part of the amplification reaction to a membrane system that contains pyrophosphatase and measuring the change in H+ concentration.* However, Bille teaches that a quantitative relationship can be obtained between pyrophosphate concentration and a change in pH inside a vesicle membrane that

contains H+-pyrophosphatase when pyrophosphate is added to a system containing vesicle membranes (claims 14, 15, and 19, see page 251, column 1, all of para 4, page 252, column 2 all of para 1, and Figures 2 and 3 of Bille). Bille teaches that the change in pH in this system is measured by a change in the absorbance of acridine orange (claims 16 and 17, see page 251, column 1, all of para 4, page 252, column 2 all of para 1, and Figures 2 and 3 of Bille). Bille also teaches that a positive current into a vacuole containing pyrophosphatase caused by a change in pH can be detected upon addition of pyrophosphate to vacuoles by the patch-clamp technique (claim 18; see page 252, column 2, all of para 4, page 253, column 1, all of para 1, and Figure 6 of Bille). Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of detecting pyrophosphate produced from the amplification step taught in the method of mutation detection of Sorenson and Kambara by subjecting the pyrophosphate to the system of vesicle membranes having pyrophosphatase and measuring the change in pH inside the vesicles or detecting such a pH change by the patch-clamp technique in view of the teachings of Bille for the purpose of developing a sensitive method of pyrophosphate detection and therefore amplification detection in the method of Sorenson and Kambara. The ordinary artisan would have a reasonable expectation of success that using the membrane associated pyrophosphatase system with a pH sensitive dye or path-clamp method taught by Bille to measure pyrophosphate levels in the method of mutation detection of Sorenson and Kambara would result in a sensitive and effective measurement of pyrophosphate, as evidenced by Figures 3 and 6 of Bille, released

during the extension reaction in the method taught by Sorenson further in view of Kambara because Bille teaches a direct quantitative relationship between pyrophosphate levels and resulting pH change in vesicle membranes as measured by a pH sensitive dye or the patch-clamp technique.

Conclusion

16. No claims are allowed.

17. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Thomas O'Farrell whose telephone number is (571) 272-8782. The examiner can normally be reached Monday-Friday from 8:30 AM to 5 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (571) 272-0745. The fax phone number for this Group is (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

Thomas O'Farrell
Examiner
Art Unit 1634

2/6/06

JEHANNE SITTON
PRIMARY EXAMINER
2/6/06